REMARKS

Reconsideration of this application is respectfully requested.

By the listing of claims included herein, claims 1 and 19-23 are canceled, claim 8 is amended, and new claims 27 and 28 are added. Support for new claim 27 is found, for example, in Figure 3. Support for new claim 28 is found, for example, at page 10, lines 31-37. No new matter is introduced.

Request for Information

The Examiner expressed concern that Applicants' reply, filed August 2, 2005, to the Request for Information Under 37 C.F.R. § 1.105, was not consistent with the abstract by Laberge et al. published in 1998 in the *European Journal of Human Genetics*, Volume 6, Supplement 1, at page 146 ("Laberge et al."), which related to a poster presented at the 30th Annual Meeting of the European Society of Human Genetics, held in Lisbon, Portugal, from May 10 to May 13, 1998.

In their previous response to the Request, Applicants submitted that there was no indication concerning the gene *Krit1* in the poster, because the *Krit1* gene had not been identified as of May, 1998.

Applicants have further considered their response, and have informed the undersigned that by their previous statement that "there was no indication concerning the gene *Krit1* in the poster, because the *Krit1* gene had not been identified as of May, 1998", they intended to convey that there is no indication concerning the fact that the presence of mutations in the *Krit1* gene are responsible for cavernous malformation, because the *Krit1* gene was not yet identified as being the CCM1 gene at that time.

Applicants also note that the sequence of the Krit 1 gene was published in Serebriiskii et al.; "Association of Krev-1/rap1a with Krit1, A Novel Ankyrin Repeat-containing Protein Encoded by a Gene Mapping to 7q21-22," *Oncogen,* Vol. 15, (1997) pp. 1043-1049. That article was disclosed to the Office in the Information Disclosure Statement filed on February 14, 2002.

Applicants believe they have fully complied with the Request for Information and request that the Examiner contact the undersigned to discuss the matter if additional information is required.

Rejection for Alleged Non-Enablement

Claims 8-16, 25, and 26 stand rejected under 35 U.S.C. § 112, first paragraph, as allegedly non-enabled. The thrust of this rejection is the Examiner's concern that not all CCM patients analyzed in the example provided in the specification display a mutation in the *Krit1* gene. Applicants note that they have amended independent claim 8 to recite "A method for genotypically diagnosing if an individual is at risk to develop cavernomas" and "said mutation giving rise to a truncated Krit1 protein, wherein said mutation is linked to the occurrence of cavernomas." Applicants believe that the amendment addresses the Examiner's concerns and submit that the amended claims are enabled in view of their disclosure.

Specifically, the inventors have identified *Krit1* as the CCM1 gene, and demonstrated that 40% of patients (8/20) belonging to families known to exhibit familial heriditary cavernomas present a truncating mutation in the Krit1 gene. In addition, the inventors have shown by SSCP analyses of the affected and unaffected members that the truncating mutations have a perfect cosegregation with the affected phenotype.

Consequently, in view of these results, the skilled artisan reading Applicants' disclosure would have understood that the claimed methods may be used for "diagnosing if an individual is at risk to develop cavernomas."

In addition, many publications since the inventors' priority application was filed, have reported that truncating mutations in the *Krit1* gene are responsible for 40 percent of familial CCM cases and that the detection of a truncating mutation in the *Krit1* gene allows an efficient (presymptomatic) molecular diagnosis of cerebral carvenous malformation. The abstracts of examples of such publications are attached hereto as Exhibit 1.

One particularly relevant abstract is that of Zawistowski et al. (2005), Hum. Mol. Gen., Vol. 14, No. 17, pp. 2521-2531), which states that familial CCM is caused by truncating mutations in KRIT1 (CCM1).

Applicants submit that Exhibit 1 demonstrates that skilled artisans have repeatedly applied their invention to practice the claimed methods, and that this demonstrates that the disclosure in this application in conjunction with the level of skill in the art is all that is required to practice the invention as claimed. Accordingly, for all of the above reasons, Applicants request that this rejection be withdrawn.

Please grant any extensions of time required to enter this response and charge any additional required fees to our Deposit Account No. 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, L.L.P.

Dated: January 24, 2007

By: _____

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ANNEXE 1

Genetics of Cavernous Malformations

By Cornelia Lee, PsyD, Judith Gault, PhD, Emily Crocker, MS, and Tracey Leedom, MS

Cerebral cavernous malformations (cavernous angiomas) can form through several different mechanisms. The major differences lie in whether you have a sporadic cavernous malformation or familial cavernous malformations.

A gene is the basic unit of heredity. Genes are made from DNA, the building block of life, and carry the information for creating the proteins which lead to a particular characteristic or function. When a gene mutates, it changes from its natural state and can cause an illness. With a sporadic cavernous malformation, it is believed that a genetic mutation has occurred in just one cell in your body. With familial cavernous malformations, a mutation of a specific gene has occurred in every cell of your body.

Sporadic Cavernous Malformation

You may have one cavernous malformation and have no other family members with the illness. It is believed that a majority of those diagnosed with the illness fall into this category. The cause of sporadic cavernous malformations is not known. However, it is thought that a solitary cavernous malformation can be formed when a single cell has two specific mutations, or changes in both copies of a particular gene. As the cell replicates and divides, it goes on to form the cavernous malformation.

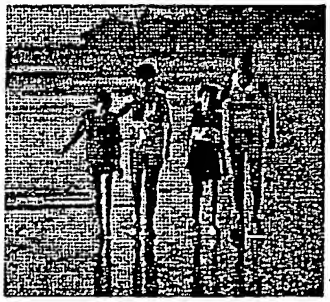
Diagnosis

To receive an accurate diagnosis of single vs. multiple cavernous angioma, it is important to use the latest technology. At present, this is MRI with gradient echo sequencing.

It is also important to have the MRI read by a physician who has experience with cavernous angioma.

A solitary cavernous malformation may be present at birth or may form later. If you have a sporadic cavernous malformation, it is likely that your children would have no greater chance of having the illness than anyone in the general population.

Familial Cavernous Malformation



Familial cavernous malformations are caused by a genetic mutation found in every cell in your body, rather than a mutation in a single cell. This illness may run in your family or you may be the first in your family to have the illness. You may have just one cavernous malformation, but are likely to have multiple cavernous malformations.

Familial cavernous malformation is a hereditary illness that is an autosomal dominant condition. This means that only one parent must have the illness for it to be passed on to offspring. Statistically, if you have the familial form of the illness and you have a child with someone who does not; your child will have a 50% chance of having the illness.

If you are the first in your family to have multiple cavernous malformations, you are likely to be the first in your family to have a familial mutation. This puts your risk of passing on the illness to your children at 50%.

ANNEXE 1 (continued)

Patient Tissue/DNA Bank & Registry
US Studies
International Studies

Resources and Links

General Resources
Disability Resources
Financial/Insurance Resources

Who We Are

About Angioma Alliance
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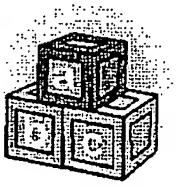
UK Resources

Familial cavernous malformations are caused by a single gene mutation in any one of three different genes. A mutation on any one of the three can cause the illness.

We each have two copies of any gene. When one copy mutates, the other is a backup that will perform the same function. However, the backup must work perfectly to avoid any problems caused by the original mutation. This is almost never the case for every cell in the body.

One theory is that in the case of familial cavernous malformation, a mutation on the first gene causes it to stop functioning. Intermittent but naturally occurring problems with the backup gene in some cells cause the formation of cavernous malformations. Wherever the backup gene fails, a cavernous malformation develops. As a result, if you have familial cavernous malformations you are likely to have more than one malformation. It is thought that almost everyone with the familial form will eventually have multiple cavernous malformations.

The Three Known Genes



To date, three genes have been linked to the familial form of the illness and have been precisely located. The first is called CCM1 (for cerebral cavernous malformation 1) and is located on chromosome 7, at 7q11.2-q21. It is also known as KRIT1, for the protein created by the gene. This is the gene responsible for most of the cases of familial multiple cavernous malformation in Hispanic families, and in a number of other families. In fact, most Hispanics with the CCM1 mutation are thought to share a common ancestor. 40% of familial cavernous malformation can be linked to a CCM1 genetic mutation.

CCM1 is responsible for creating KRIT1 protein, or Krev interaction-trapped 1 protein. This protein is considered to be important for basic life development. The exact function of KRIT1 protein is not known but it is believed to play a role in determining the structure of epithelial cells in blood vessels in the brain. When the first copy of the CCM1 gene mutates, only the backup copy can produce KRIT1 protein. If there are problems with the second copy of the gene, the KRIT1 protein can not function and cavernous malformations form.

The second gene is called CCM2. It is located at 7p15-p13 and controls the production of a protein named malcavernin. The malcavernin protein is believed to play a role in determining where in a vascular epithelial cell (nucleus versus cytoplasm) KRIT1 will be active. When it mutates, too much KRIT1 is in the nucleus of the cell and not enough is in the cytoplasm. 20% of familial cavernous malformation can be linked to a CCM2 mutation.

The third gene, CCM3, is on the 3rd chromosome at 3q26.1. CCM3 is responsible for creating a protein called Programmed Cell Death 10 or PDCD10. As of July, 2005, the function of this protein in the formation of cavernous malformations is not known.

For more information on these three genes, please visit the Genetics Home Reference. Genetics Home Reference is a service of the National Library of Medicine. These are the links:

CCM1 (KRIT1): http://ghr.nlm.nih.gov/gene=krit1
CCM2 (malcavernin): http://ghr.nlm.nih.gov/gene=ccm2
CCM3 (PDCD10): http://ghr.nlm.nih.gov/gene=pdcd10

Genetic Testing

Clinical genetic testing, the only kind of testing that can be used for diagnosis, is available for all three currently known genes. See our Genetic Testing page to find specific laboratories that have been approved to perform these tests.

ANNEXE 2 (continued)

Because not all of the genes have been identified, genetic testing can not rule out a familial mutation. However, if a mutation is identified, it becomes very easy and economical to test other family members.

Whether to have genetic testing is a very personal decision. Please make sure that you have a knowledgeable genetic counselor or physician to help guide you.

Current Research

Many researchers working on cavernous malformations are focused on genetic issues. It seems to hold the most promise for future understanding and eventual cure. The current focus is on identifying the precise functions of the proteins created by the genes. Please follow the Research Studies link to see a summary of the major laboratories and their ongoing work in this area. Also, please see our Latest Research section and our newsletter for more detailed information about recent genetic discoveries in this area.

Links

To find general information on genetics, visit GeneTests or the Genetics Home Reference.

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CCM1 and CCM2 protein interactions in cell signaling: implications for cerebral cavernous malformations pathogenesis.
Hum Mol Genet. 2005 Jul 21; [Epub ahead of print].

Genes >

KRIT1

Print version

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Gene Reviews ⇒
Clinical summary

Gene Tests ⇒
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Research Resources
Tools for researchers

What is the official name of the KRIT1 gene?

The official name of this gene is "KRIT1, ankyrin repeat containing."

KRIT1 is the gene's official symbol. The KRIT1 gene is also known by other names, listed below.

What is the normal function of the KRIT1 gene?

The KRIT1 gene (also known as the CCM1 gene) provides instructions for making a protein that likely plays an important role in the formation of blood vessels, especially capillaries, during the development of an embryo. While the exact function of the KRIT1 protein is not clearly understood, studies suggest that it influences the movement and structure of cells during the development of blood vessels. The KRIT1 protein may also be involved in creating the boundary between the walls of capillaries and the surrounding tissue in the brain, called the blood-brain barrier. This barrier protects the brain's delicate nerve tissue by preventing many types of molecules from entering the brain. Research suggests that the KRIT1 protein may also help maintain the structure and function of blood vessels after they have formed.

What conditions are related to the KRIT1 gene?

cerebral cavernous malformation - caused by mutations in the KRIT1 gene

More than 100 mutations that cause cerebral cavernous malformations have been identified in the KRIT1 gene. Virtually all of these mutations place a premature stop signal in the instructions for making the KRIT1 protein, preventing adequate KRIT1 protein production. Without enough KRIT1 protein, blood vessels do not form properly and cavernous malformations can develop.

→ (Mutations in the KRIT1 gene may account for up to 40 percent of all familial cerebral cavernous malformation cases. One particular mutation is responsible for up to 70 percent of cerebral cavernous malformation cases in people of Hispanic heritage. This mutation replaces one DNA building block (nucleotide base) with a different base at position 1363 in the KRIT1 gene, written as 1363C>T.

Where is the KRIT1 gene located?

Human Molecular Genetics, 2005, Vol. 14, No. 17 2521–2531
doi:10.1093/hmg/ddi256
Advance Access published on July 21, 2005

CCM1 and CCM2 protein interactions in cell signaling: implications for cerebral cavernous malformations pathogenesis

Jon S. Zawistowski[1,†], Lisa Stalheim[2,†], Mark T. Uhlik[2], Amy N. Abell[2], Brooke B. Ancrile[1], Gary L. Johnson[2] and Douglas A. Marchuk[1,*]

[1]Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC 27710, USA and [2]Department of Pharmacology and the Lineberger Comprehensive Cancer Center, University of North Carolina School of Medicine, Chapel Hill, NC 27599-7365, USA

Received April 28, 2005; Revised and Accepted July 14, 2005

Cerebral cavernous malformations (CCMs) are sporadically acquired or inherited vascular lesions of the central nervous system consisting of clusters of dilated thin-walled blood vessels that predispose individuals to seizures and stroke. Familial CCM is caused by mutations in KRIT1 (CCM1) or in malcavernin (CCM2), the murine ortholog of which was concurrently characterized as osmosensing scaffold for MEKK3 (OSM). The roles of the CCM proteins in the pathogenesis of the disorder remain largely unknown. Here, we use co-immunoprecipitation, fluorescence resonance energy transfer and subcellular localization strategies to show that the *CCM1* gene product, KRIT1, interacts with the *CCM2* gene product, malcavernin/OSM. Analogous to the established interactions of CCM1 and β_1 integrin with ICAP1, the CCM1/CCM2 association is dependent upon the phosphotyrosine binding (PTB) domain of CCM2. A familial CCM2 missense mutation abrogates the CCM1/CCM2 interaction, suggesting that loss of this interaction may be critical in CCM pathogenesis. CCM2 and ICAP1 bound to CCM1 via their respective PTB domains differentially influence the subcellular localization of CCM1. Furthermore, we expand upon the established involvement of CCM2 in the p38 mitogen-activated protein kinase signaling module by demonstrating that CCM1 associates with CCM2 and MEKK3 in a ternary complex. These data indicate that the genetic heterogeneity observed in familial CCM may reflect mutation of different molecular members of a coordinated signaling complex.

INTRODUCTION

Cerebral cavernous malformations (CCMs) are vascular lesions of the central nervous system consisting of clusters of dilated thin-walled blood vessels or 'caverns'. The cluster of vessels that defines the CCM lesion is surrounded by connective tissue while remaining distinct from the surrounding neural parenchyma (1). The cavernous malformation lesions have the capacity to hemorrhage, resulting in seizures, stroke and focal neurological deficits (2,3).

Three forms of autosomal dominant CCM have been mapped (4–7), and the disease gene products are known for all three of the mapped loci. *CCM1* is caused by truncating mutations in KRIT1 (8,9), a protein containing ankyrin repeat motifs and a FERM domain. *CCM2* results from mutations in *MGC4607* (10,11), encoding the phosphotyrosine binding (PTB) domain protein malcavernin (10), the murine ortholog of which was characterized as a mitogen-activated protein kinase (MAPK) scaffold named osmosensing scaffold for MEKK3 (OSM) (12). *CCM3* has recently been shown to result from mutations in *PDCD10* (programmed cell death 10), a gene upregulated in the human myeloid cell line TF-1 upon induction of apoptosis (13). Germline mutations within *CCM1* kindreds are primarily nonsense, frameshift or splice site mutations, predicted to be loss of function alleles (14). A similar class of mutations is found in *CCM2* and *CCM3* pedigrees (10,11,13); however, a missense mutation has been reported for a *CCM2* kindred (11).

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†The authors wish it to be known that, in their opinion, the first two authors should be regarded as joint First Authors.

7,1P

1: Zhonghua Bing Li Xue Za Zhi. 2003 Jun;32(3):220-5.

Links

[A novel Krit-1 mutation in Han family with cerebral cavernous malformation]

[Article in Chinese]

Xu YL, Zhao JZ, Wu BQ, Zhong HH, Wang S, Heng WJ.

Department of Neurosurgery, Tiantan Hospital Affiliated to Capital University of Medical Sciences, Beijing 100050, China.

OBJECTIVE: To detect the mutations of Krit-1 gene that cause familial cerebral cavernous malformation (CCM) in the Han ethnic origin. METHODS: The subjects were hospitalized in the Department of Neurosurgery, Tiantan Hospital affiliated to Capital University of Medical Sciences. Two families (A and B) and 8 apparently sporadic individuals affected with CCM were screened for mutations of Krit-1 gene. Members of the family CCM have a wide range in age of onset with seizures, headaches and skin lesions. The gene was screened by PCR amplification of 16 exons and mutation was detected by direct sequencing. RESULTS: In family A samples, analysis of the Krit-1 gene revealed a new point mutation in exon 14 [a heterozygous C to G transition at nucleotide 1 289 (counting from the start codon or nt 2 308 counting from the first nt of the mRNA, aligned according to Gene Bank AF388384)] which predicts the substitution of a premature termination codon for Serine at codon 430 (S430X), belonging a nonsense point mutation. No mutation was identified in one of family A members as well as in any of the sporadic individuals with the exception of a single nucleotide polymorphism. CONCLUSIONS: Report the first family in the Han with CCM having a novel mutation in the CCM1 gene on the continent of Asia. The newly identified mutation creates a premature termination codon and is predicted to produce a truncated Krev1 interaction-trapped 1 protein, KRIT1. This result allows efficient presymptomatic molecular diagnosis.

PMID: 12882686 [PubMed - indexed for MEDLINE]

1: Proc Natl Acad Sci U S A. 2002 Aug 6;99(16):10677-82. Epub 2002 Jul 24.

KRIT1, a gene mutated in cerebral cavernous malformation, encodes a microtubule-associated protein.

Gunel M, Laurans MS, Shin D, DiLuna ML, Voorhees J, Choate K, Nelson-Williams C, Lifton RP.

Department of Neurosurgery, Yale Neurovascular Surgery Program, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06510, USA.

Mutations in Krev1 interaction trapped gene 1 (KRIT1) cause cerebral cavernous malformation, an autosomal dominant disease featuring malformation of cerebral capillaries resulting in cerebral hemorrhage, strokes, and seizures. The biological functions of KRIT1 are unknown. We have investigated KRIT1 expression in endothelial cells by using specific anti-KRIT1 antibodies. By both microscopy and coimmunoprecipitation, we show that KRIT1 colocalizes with microtubules. In interphase cells, KRIT1 is found along the length of microtubules. During metaphase, KRIT1 is located on spindle pole bodies and the mitotic spindle. During late phases of mitosis, KRIT1 localizes in a pattern indicative of association with microtubule plus ends. In anaphase, the plus ends of the interpolar microtubules show strong KRIT1 staining and, in late telophase, KRIT1 stains the midbody remnant most strongly; this is the site of cytokinesis where plus ends of microtubules from dividing cells overlap. These results establish that KRIT1 is a microtubule-associated protein; its location at plus ends in mitosis suggests a possible role in microtubule targeting. These findings, coupled with evidence of interaction of KRIT1 with Krev1 and integrin cytoplasmic domain-associated protein-1 alpha (ICAP1 alpha), suggest that KRIT1 may help determine endothelial cell shape and function in response to cell-cell and cell-matrix interactions by guiding cytoskeletal structure. We propose that the loss of this targeting function leads to abnormal endothelial tube formation, thereby explaining the mechanism of formation of cerebral cavernous malformation (CCM) lesions.

PMID: 12140362 [PubMed - indexed for MEDLINE]

1: J Neurosurg. 2004 May;100(5 Suppl Pediatrics):481-7.

Links

Krev1 interaction trapped-1/cerebral cavernous malformation-1 protein expression during early angiogenesis.

Guzeloglu-Kayisli O, Kayisli UA, Amankulor NM, Voorhees JR, Gokce O, DiLuna ML, Laurans MS, Luleci G, Gunel M.

Yale Neurovascular Surgery Program and Department of Neurosurgery, Yale University School of Medicine, New Haven, Connecticut, USA.

OBJECT: Molecular genetic studies of cerebral cavernous malformation (CCM) have identified three loci, CCM1-3, that can lead to CCM when mutated. Examination of the CCM1 locus established KRIT1 (Krev1 Interaction Trapped genre 1) as the CCM1 gene. Despite the identification of KRIT1 as the gene mutated in CCM1, little has been learned regarding its function. The authors recently demonstrated specific KRIT1 expression in endothelial cells. Based on this result and the fact that the CCM phenotype features defects in microvasculature, we hypothesized that KRIT1 may take an active part in normal angiogenesis. METHODS: In this study, the authors investigated the spatial and temporal expression of KRIT1 during normal vessel development and maturation by examining KRIT1 protein in both in vitro and in vivo angiogenic systems with the use of postconfluent endothelial cell cultures along with placental tissues from different developmental stages. CONCLUSIONS: The results demonstrate that KRIT1 is expressed during capillary-like tube formation in the early stages of angiogenesis in vitro. Histological examination of placental tissue, a well-established in vivo model of angiogenesis, shows KRIT1 expression in active angiogenic and vasculogenic areas of the immature placental villi. As the placenta matures, KRIT1 expression is restricted to microvascular and small arterial endothelial cells with little or no expression seen in the intima of large vessels. It can therefore be concluded that KRIT1 is expressed during early angiogenesis by endothelial cells and may play a key role in vessel formation and/or development.

PMID: 15287459 [PubMed - indexed for MEDLINE]

Related Links

KRIT1, a gene mutated in cerebral cavernous malformation, encodes a microtubule-associated protein. [Mol Cell Biol. 2002]

Interaction between krit1 and icap1alpha infers perturbation of integrin beta1-mediated angiogenesis in the pathogenesis of cerebral cavernous malformation. [Hum Mol Genet. 2001]

Mutation and expression analysis of the KRIT1 gene associated with cerebral cavernous malformations (CCM1). [Acta Neuropathol (Berl). 2002]

Identification of Krit1B: a novel alternative splicing isoform of cerebral cavernous malformation gene-1. [Gene. 2004]

Krit1/cerebral cavernous malformation 1 mRNA is preferentially expressed in neurons and epithelial cells in embryo and adult. [Mech Dev. 2002]

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Dec 18 2006 06:34:27

1: J Neurol Sci. 2002 Apr 15;196(1-2):91-6. ELSEVIER FULL TEXT ARTICLE Links

Cerebral cavernous malformation: novel mutation in a Chinese family and evidence for heterogeneity.

Chen DH, Lipe HP, Qin Z, Bird TD.

Department of Neurology, University of Washington, Seattle, WA, USA.

Familial cerebral cavernous malformation (CCM) is an autosomal dominant disorder producing vascular anomalies throughout the central nervous system associated with seizures and hemorrhagic stroke. Linkage analysis has shown evidence for at least three genetic loci underlying this disorder with a founder mutation in the Mexican/Hispanic community. We report the first family of Chinese ethnic origin with CCM having a novel mutation in the CCM1 gene. The mutation in exon 19 causes a premature stop codon (Q698X) predicted to produce a truncated Krev1 interaction-trapped 1 (KRIT1) protein. Members of the family with this mutation have a wide range in age of onset with seizures, ataxia, spinal cord vascular malformation, headaches and skin lesions. An additional unrelated sporadic subject with brain lesions compatible with CCM as well as vascular skin findings suggesting the blue rubber bleb nevus (BRBN) syndrome has no mutation detected in the CCM1 gene. These findings expand the phenotype of and demonstrate further evidence for the heterogeneity in the CCM syndrome.

PMID: 11959162 [PubMed - indexed for MEDLINE]

1: J Neurol Sci. 2003 Aug 15;212(1-2):75-8.

ELSEVIER FULL TEXT ARTICLE Links

Identification of a novel KRIT1 mutation in an Italian family with cerebral cavernous malformation by the protein truncation test.

Marini V, Ferrera L, Dorcaratto A, Viale G, Origone P, Mareni C, Garre C.

Department of Oncology, Biology and Genetics, University of Genova, Viale Benedetto XV, 6, Genoa 16132, Italy.

Familial cerebral cavernous malformation (CCM) exhibits autosomal dominant inheritance and is characterized by vascular disorders of the brain, which can lead to seizures, focal neurological deficits, hemorrhagic stroke, and migraine. Three CCM loci have been mapped, but the gene for only one locus--KRIT1 coding for Krev-1/rap1 interaction trapped 1 (KRIT1) protein, which is responsible for more than 40% of familial cases--has been identified. To date, a total of 72 mutations have been described, with one founder effect in the Mexican/Hispanic community. We report the case of an Italian family with CCM that has a novel KRIT1 gene mutation leading to a truncated KRIT1 protein. The protein truncation test (PTT) has been used as a rapid method of identifying germline mutations in the KRIT1 gene.

PMID: 12810002 [PubMed - indexed for MEDLINE]

1: Eur J Hum Genet. 2002 Nov;10(11):733-40.

Spectrum and expression analysis of KRIT1 mutations in 121 consecutive and unrelated patients with Cerebral Cavernous Malformations.

Cave-Riant F, Denier C, Labauge P, Cecillon M, Maciazek J, Joutel A, Laberge-Le Couteulx S, Tournier-Lasserve E.

INSERM EMI 99-21, Faculte de Medecine Lariboisiere, Paris, France.

Cerebral Cavernous Malformations (CCM/MIM 604214) are vascular malformations characterised by abnormally enlarged capillary cavities without intervening brain parenchyma. Clinical manifestations include seizures, cerebral haemorrhages and focal neurological deficits. They occur as a sporadic or autosomal dominant condition. Most often, sporadic cases have only one lesion and familial cases are characterised by a high frequency of multiple lesions. Three CCM loci were previously mapped on 7q (CCM1), 7p (CCM2) and 3q (CCM3) and CCM1 gene was identified as coding Krit1, a protein of unknown function, which was shown initially to interact in yeast two hybrid assays with Rap1A, a small ras GTPase and more recently to Icap1alpha, a modulator of beta1 integrin signal transduction. Herein, we screened KRIT1 gene in 121 unrelated, consecutively recruited, CCM probands having at least one affected relative and/or showing multiple lesions on cerebral MRI. Fifty-two of these probands (43%) were shown to carry a KRIT1 mutation. Forty-two distinct mutations were identified including six recurrent ones. Three-quarters of these mutations were located in the C-terminal half of the gene, mostly within exons 13, 15 and 17. All of them are predicted to lead to a premature stop codon. No missense mutation was identified. The only two nucleotide substitutions predicted to be missense mutations led in fact to an abnormal splicing and a premature stop codon. Altogether these data suggest that KRIT1 mRNA decay due to the presence of premature stop codons and Krit1 haploinsufficiency may be the underlying mechanism of CCM.

PMID: 12404106 [PubMed - indexed for MEDLINE]

1: Neurosurgery. 2000 May;46(5):1272-7; discussion 1277-9.

Mutations in KRIT1 in familial cerebral cavernous malformations.

Zhang J, Clatterbuck RE, Rigamonti D, Dietz HC.

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OBJECTIVES: The recognition of six unrelated Hispanic-American families in which cerebral cavernous malformations (CCM) segregated as an autosomal dominant trait established a genetic basis for this disease. Linkage analysis subsequently identified locus heterogeneity with disease genes for CCM at chromosomal regions 7q, 7p, and 3q. Recently, mutations in KRIT1, a gene on 7q at the CCM1 locus, were identified in French and Hispanic-American families with CCM. This study confirms the identity the KRIT1 founder mutation in Hispanic-Americans and reports a novel KRIT1 mutation in a Caucasian family. METHODS: Oligonucleotide primers were designed to allow amplification of genomic DNA sequences from four Hispanic-American families and five non-Hispanic families for all 12 exons of the KRIT1 gene using the polymerase chain reaction (PCR). The amplified DNA was then screened using single strand conformation polymorphism analysis (SSCP) and sequencing. The expression pattern of KRIT1 was analyzed by Northern blotting. RESULTS: Analysis of the KRIT1 gene revealed a point mutation in exon 6 that predicts the substitution of a premature termination codon for glutamine at codon 248 in all four Hispanic-American families, confirming previous findings. SSCP analysis and sequencing revealed an 11 base pair duplication in exon 7 leading to a premature termination codon in one Caucasian family. Northern analysis demonstrated widespread expression of this gene, however, the highest level of expression was in the brain. CONCLUSION: The common KRIT1 mutation causing the majority of CCM in Hispanic-Americans has been identified and independently confirmed, allowing efficient presymptomatic molecular diagnosis. In keeping with prior results, both newly identified mutations create a premature termination codon and are predicted to initiate degradation of the mutant mRNA through the nonsense-mediated mRNA decay pathway. These data strongly suggest loss of function as the relevant patho-genetic mechanism.

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Cerebral cavernous malformations: mutations in Krit1.

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OBJECTIVE: To find mutations in the recently identified additional exons of the Krit1 gene that causes CCM1, a disease characterized by the formation of cerebral cavernous malformations (CCM). To determine the relative frequency with which Krit1 mutations cause CCM as well as recharacterize the mutations reported in the literature. METHODS: Twenty-seven families and 11 apparently sporadic individuals affected with CCM were screened for mutations in the Krit1 gene. The gene was screened by single stranded conformation polymorphism, and variants were sequenced. Familial segregation of the mutations was determined. RESULTS: In familial samples, two new mutations in the novel upstream exons and six additional mutations in the previously identified exons were identified. No mutation was found in any of the sporadic individuals. CONCLUSIONS: Results demonstrate that the frequency of mutations found in Krit1 is 47% in the families studied and the frequency may increase as more mutations are detected. Mutations are evenly distributed in the gene and do not seem to be limited to structural domains present in Krit1. This is in accordance with the model that Krit1 could be a tumor suppressor gene.

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